Influence of electron acceptor availability and microbial community structure on sedimentary methane oxidation in a boreal estuary

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Abstract Methane is produced microbially in vast quantities in sediments throughout the world's oceans. However, anaerobic oxidation of methane (AOM) provides a near-quantitative sink for the produced methane and is primarily responsible for preventing methane emissions from the oceans to the atmosphere. AOM is a complex microbial process that involves several different microbial groups and metabolic pathways. The role of different electron acceptors in AOM has been studied for decades, yet large uncertainties remain, especially in terms of understanding the processes in natural settings. This study reports

whole-core incubation methane oxidation rates along an estuarine gradient ranging from near fresh water to brackish conditions, and investigates the potential role of different electron acceptors in AOM. Microbial community structure involved in different methane processes is also studied in the same estuarine system using high throughput sequencing tools. Methane oxidation in the sediments was active in three distinct depth layers throughout the studied transect, with total oxidation rates increasing seawards. We find extensive evidence of non-sulphate AOM throughout the transect. The highest absolute AOM rates were observed below the sulphate-methane transition zone (SMTZ), strongly implicating the role of alternative electron acceptors (most likely iron and manganese oxides). However, oxidation rates were ultimately limited by methane availability. ANME-2a/b were the most abundant microbial phyla associated with AOM throughout the study sites, followed by ANME-2d in much lower abundances. Similarly to oxidation rates, highest abundances of microbial groups commonly associated with AOM were found well below the SMTZ, further reinforcing the importance of non-sulphate AOM in this system.

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Introduction

Methane (CH₄) is a powerful greenhouse gas affecting the global climate. Its atmospheric concentrations have more than doubled since the industrial revolution, due to anthropogenic activities (IPCC 2014). In aquatic systems, production of CH₄ primarily takes place in sediments through methanogenesis, which is the final step in anaerobic breakdown of organic matter that occurs when other electron acceptors (EA) have been depleted and carbon dioxide (CO₂) is the only viable electron acceptor remaining (Thauer 1998). The exact sediment depth of the primary methanogenic zone depends on the organic matter loading of the system, spanning from a few centimeters in productive coastal systems to several meters in the oligotrophic open ocean seabed (Jørgensen et al. 2001). Methanogenesis is also typically more active in freshwater sediments than in marine sediments, due to the presence of sulphate (SO₄^{2−}) in seawater (Capone and Kiene 1988), which provides a more energetically favorable pathway for anaerobic remineralization. Eutrophication is expected to increase methanogenesis globally due to enhanced carbon loading (Beaulieu et al. 2019).

Although CH₄ is produced in sediments in large quantities, as a highly reduced compound it is susceptible to microbial oxidation. These oxidative processes create a “filter” which prevent CH₄ from escaping to the atmosphere (Knittel and Boetius 2009). In aquatic systems, CH₄ is oxidized through both oxic and anoxic processes. Of these, aerobic oxidation of methane (MOX) is typically more prevalent in fresh waters (de Angelis and Scranton 1993). It is typically most prominent at steep oxyclines, such as the pycnocline of a stratified water column (Schmale et al. 2010; Jakobs et al. 2013) or the sediment–water interface (Fenchel et al. 1995).

Unlike MOX, anaerobic oxidation of methane (AOM) tends to be more efficient at higher salinities, due to increased availability of SO₄^{2−} in seawater (Knittel and Boetius 2009). Of these two processes, AOM is thus the major sink of CH₄ in marine systems, though extreme variability in process rates exists. Globally, AOM has been shown to be a near-quantitative sink for CH₄ produced by sedimentary methanogenesis (Egger et al. 2018). It was originally thought that due to its SO₄^{2−}-dependence, AOM is an exclusively marine process, but it has since been

shown to be active also in lakes (Eller et al. 2005; Sivan et al. 2011; Martinez-Cruz et al. 2018).

SO₄^{2−} mediated AOM (S-AOM) is mainly performed by anaerobic methanotrophic (ANME) archaea with sulfate reducing bacteria (SRB) via direct interspecies electron transfer (McGlynn et al. 2015). It is the most important AOM reaction in marine systems (Knittel and Boetius 2009) and the stoichiometry has been formulated as follows (Hoehler et al. 1994):

It has later been established that at least nitrite/nitrate (Ettwig et al. 2010; Timmers et al. 2017), as well as oxides of iron (Fe, Egger et al. 2015; Ettwig et al. 2016) and manganese (Mn, Beal et al. 2009) can also be used as alternatives to SO₄^{2−} as terminal electron acceptors by the different ANME clades.

Iron mediated AOM (Fe-AOM):

Manganese mediated AOM (Mn-AOM):

Fe-AOM requires the concurrent presence of CH₄ and reducible iron (i.e. Fe(OH)₃) below the sulphate-methane transition zone (SMTZ). This is possible either under sedimentation regimes with high Fe(OH)₃ deposition or recent shoaling of the SMTZ due to carbon loading, both of which are typical in eutrophied coastal systems (Rooze et al. 2016; Jilbert et al. 2018). Most microbes capable of Fe reduction can also reduce Mn and may do so preferentially due to the higher energy yield of the process (Lovley and Phillips 1988). Beal et al. (2009) showed that though Mn-AOM exhibits slower process rates compared to SO₄^{2−}-AOM, energetically Mn-AOM can be up to 10 times more favorable. Mn-AOM and Fe-AOM will be collectively referred to as Me-AOM henceforth in this paper.

Many methanotrophic archaea are capable of AOM. These have been divided into three main groups and a number of subclusters: ANME-1 (subgroups a and b), ANME-2 (subgroups a, b, c and d, of which the two latter are distinct from the two former, and also from each other) and ANME-3 (Haroon et al. 2013; Ettwig et al. 2016; Timmers et al. 2017). Outside the ANME, a group of bacteria called *Candidatus* Methyloirambis

(in phylum NC10) are capable of AOM (Ettwig et al. 2010). It has been suggested that the main biochemical pathway of CH_4 oxidation in ANME is the enzymatic reversal of methanogenesis (McGlynn 2017). However, complexity arises from the fact that several of the ANME clades have been shown to have the capacity also for methanogenesis (Ding et al. 2016).

Understanding AOM, and the role of different compounds as electron acceptors is important, not only because of the global warming potential of CH_4 , but also because of the influence of AOM on other biogeochemical cycles, such as those of phosphorus (P), sulphur (S), Fe and Mn. As an example, Fe-AOM and Mn-AOM could potentially lead to increases in sediment release and subsequent lateral transfer (“shuttling”) of Fe, Mn and P from the coastal zone to offshore regions in systems such as the Baltic Sea (Jilbert and Slomp 2013; Reed et al. 2016; Rooze et al. 2016). In this study our aim was to investigate the factors controlling sedimentary AOM rates along an estuarine gradient in the northern Baltic Sea. To do this, we conducted full-core incubations on sediments from three sites in the estuary, spanning large vertical and lateral gradients in potential electron acceptor availability, thus allowing us to investigate the influence of electron acceptors on methane oxidation rates. In addition to the oxidation rate measurements, we make use of sediment microbial community compositional data from a parallel study in the same estuarine system, in order to investigate the key microbial groups involved in CH_4 cycling. We hypothesize that the estuarine gradient is characterized by increasing availability of sulfate offshore, and that this in turn determines the depth of the SMTZ and vertical distribution of methane oxidation zones and related microbial communities in the sediments.

Materials and methods

Study area

Samples were collected along a transect in the Pojo Bay estuary, located in Southwestern Finland, from four sites: A, C, D and J (Fig. 1). The non-sequential lettering for the sites is used to maintain compatibility with a companion paper detailing the wider CH_4 dynamics in the same system (Myllykangas et al. 2020). The sites span a water column salinity gradient of 0–7.

Site A is located in the river mouth of River Karjaa, which is the primary freshwater source of the estuary. Site A is permanently oxic, with bottom water salinity 0–2, and receives organic carbon of primarily terrestrial origin (Jilbert et al. 2018). Due to its proximity to rivermouth flocculation of Fe, from both natural and anthropogenic sources, site A also has strongly elevated concentrations of Fe in the sediments (up to 10–20% by weight). Approximately half of the sedimentary Fe is expected to be in reducible oxide forms as determined by sequential extraction (Jilbert et al. 2018).

Sites C and D are in the main depression of the inner bay (bottom water salinity 3–5), which is separated from the connecting archipelago by a narrow sill close to the city of Ekenäs. These sites experience seasonal hypoxia and sediment focusing, and are subject to relatively intense carbon accumulation from both terrestrial and phytoplankton sources (Jilbert et al. 2018). These sites have intermediate sedimentary Fe concentrations, but are strongly enriched in Mn due to redox shuttling of Mn-oxides during seasonal hypoxia in the inner bay (Tiihonen 2016).

Site J is seawards in the archipelago (bottom water salinity 6–7) along the main channel of the estuary. It is typically fully oxic and receives primarily phytoplankton-derived organic carbon (Jilbert et al. 2018). The sediments contain lower concentrations of Fe and Mn than those at sites A, C and D, but concentrations are nevertheless significantly elevated (e.g. Fe = 6–7%) with respect to sites offshore in the Baltic Sea (Jilbert et al. 2018).

In coastal sediments of the Gulf of Finland generally, bioturbation is known to be important at oxic sites but restricted at sites showing seasonal hypoxia (Gammal et al. 2017; Kauppi et al. 2018). Hence, of our sites, A and J are expected to show intense bioturbation, while sites C and D are expected to be relatively undisturbed by benthic fauna.

Sediment sampling for incubation experiments and pore water profiling

Sediment was retrieved using a GEMAXTM—twin corer in August 2017 from sites A, C, and J on board R/V *Saduria*. Four acrylic minicores (26 ID × 400 mm) were taken from each site for incubation (three replicates and one control). During transport, minicores were capped from both ends and stored upright submersed in site bottom water at in situ temperature.

Fig. 1 Map of the Pojo Bay estuary and the connected archipelago. Sites A and D (cross) were sampled for microbial data, sites A, C and J (circle) for oxidation rate

Samples for vertical porewater CH_4 profiles were measured from a parallel core directly following retrieval. 10 ml of wet sediment was extracted at 2.5 cm intervals using cutoff plastic syringes. Sediment was immediately transferred into 65 mL glass bottles pre-loaded with supersaturated NaCl solution, which were immediately capped with butyl rubber septa and screw caps and stored upside down. Within 24 h, 10 mL of N_2 headspace was injected by needle through the stopper, and an equivalent volume of sediment slurry removed through a second needle. After equilibration, two 1 mL subsamples of the headspace were analyzed with a gas chromatograph equipped with a flame ionization detector (Agilent Technologies 7890B). Sediment volume in the original sample was calculated from complementary porosity profiles.

One additional GEMAX-core was retrieved per site and sampled at 1 cm intervals with RhizonsTM for

porewater extraction and later analyzed for Fe, Mn, S and Ca with inductively coupled plasma—optical emission spectrometry (ICP-OES). The samples were acidified with 10 μL of 65% HNO_3 per mL of porewater in order to keep metals in solution. ICP-OES-derived S is interpreted to represent SO_4^{2-} , since H_2S is lost upon the addition of HNO_3 . The analyzed metals are interpreted to be present as divalent ions. For full details see Jilbert et al. (2018).

Determination of methane oxidation rates

Incubation

Each of the minicores was pre-drilled with holes at 1 cm intervals on the side. The holes were plugged with silicone and taped from both sides with water resistant tape. In the lab, a 25 μL of 0.25 KBq activity $^{14}\text{CH}_4$ (Biotrend Chemikalien) label (2.26 nmol)

dissolved in oxygen-free artificial seawater was injected into the cores through the side ports with a gastight glass syringe (Hamilton). The needle was first pushed through the core horizontally and then simultaneously withdrawn during the injection, in order to ensure even distribution of the tracer. The cores were incubated without top caps in a temperature-controlled water bath at in situ temperature for 24 h.

The incubation was stopped and microbial activity was halted by sectioning the cores at 2 cm intervals (total volume per slice approximately 11 cm³) and funneling the slices into pre-weighed 100 mL glass bottles containing 40 mL of 2.5% NaOH. Following the NaOH addition, pH in the slurry was consistently > 12, ensuring that all DIC in the slurry was present as carbonate (CO₃²⁻). The bottles were immediately closed with a septum cap (PFTA, 5 mm) and stored upside down.

Analysis

The total amount of CH₄ in the samples after incubation and storage was calculated from a 1 mL subsample taken from the headspace through the septum cap, which was subsequently analyzed with a gas chromatograph equipped with a flame ionization detector (Agilent Technologies 7890B). To capture residual ¹⁴CH₄ tracer not converted to ¹⁴CO₂ during incubation, the headspace of samples was purged with synthetic air through a glass tube containing copper oxide and combusted in a tube oven at 850 °C. The combusted ¹⁴CO₂ was trapped in a 20 mL scintillation vial containing 10 mL of CO₂-absorbant (2-phenylethylamine and 2-methoxyethanol, 1:7 v/v). After 15 min of purging, 10 mL of Ultima GoldTM scintillation cocktail (Perkin Elmer) was added and samples were subsequently analyzed using a liquid scintillation counter (Wallac® 1415). The bottles were weighed again to assess total sediment volume of each sample.

Following combustion, the slurry jars were left uncapped for 10 min to allow all residual CH₄ to escape. Subsequently, a 20 mL glass scintillation vial containing CO₂-absorbant (6 mL of 0.5 M NaOH and 2-phenylethylamine solution, 1:1 v/v) was gently inserted into the sediment slurry bottle. The slurry was then acidified with 12 mL of 6 M HCl injected to the bottom of the slurry and capped rapidly. The acidification procedure lowered the pH of the slurry

to < 1, which caused the release of all DIC in the slurry as CO₂, which was subsequently trapped in the amine solution in the headspace. After 12 h under gentle magnetic stirring, the scintillation vials were removed and wiped thoroughly with ethanol, after which 10 mL of scintillation liquid was added, and the samples were analyzed for ¹⁴CO₂ with a liquid scintillation counter.

Controls

One full minicore from each site was dedicated as a control and treated the same way as the replicates, except that the tracer was added only after the core had been sliced and fixed in NaOH. Controls were analyzed from five different depths from each site. The volume of injected tracer was identical to that during the sample incubations.

Rate calculations

In situ rates of AOM were calculated from the fraction of produced ¹⁴CO₂ in the total ¹⁴C pool using Eq. 1 (modified from Treude et al. 2005):

$$AOM = \frac{{}^{14}\text{CO}_2 \times \text{CH}_4}{({}^{14}\text{CH}_4 + {}^{14}\text{CO}_2) \times t \times v} \quad (1)$$

where ¹⁴CO₂ and ¹⁴CH₄ are the activities (disintegrations per minute, DPM) of microbially produced CO₂ during incubation and the activity of the injected CH₄ not incorporated into CO₂, respectively, CH₄ is the total amount of CH₄ in the sample in nmol at time zero, *t* is the incubation time in days and *v* volume of slices in cm³. Average of DPM values from the blanks were subtracted from the ¹⁴CO₂ values, and only samples with activity more than 3 × SD of the blanks were considered active. The DPM values of ¹⁴CH₄ were corrected with the ratio between T₀ and post-incubation CH₄ concentrations to account for losses of CH₄ during storage, which are assumed to occur at the same rate for both ¹⁴CH₄ and ¹²CH₄ (Treude et al. 2005). Total ¹⁴C label recovered in both combustion and acidification steps was 85–95%.

Depth-integrated oxidation rates were calculated from the mean oxidation rates between 0 and 31 cm depths utilizing the trapezoid rule:

$$\int_{31\text{cm}}^{0\text{cm}} f(x)dx \approx \frac{1}{2} \sum_{k=1}^N (x_{k+1} - x_k) (f(x_{k+1}) + f(x_k)) \quad (2)$$

where $f(x)$ is oxidation rate in $\text{mmol m}^{-3} \text{ day}^{-1}$, x is the sediment depth zone in cm.

Microbial communities

Analysis of bacterial and archaeal community composition

Samples for community composition analysis were collected in June 2015 from sites A and D with a GEMAXTM corer. This work was carried out as part of a parallel project, and data was made available to the current study. The presence of a well-defined shallow (5–15 cm) SMTZ at all muddy sites in the Pojo Bay system (Myllykangas et al. 2020) facilitates qualitative comparison of the vertical structure of the microbial communities and CH_4 oxidation rates, regardless of spatial offset in sampling locations.

Sediment was sliced at 2.5 cm intervals and stored in 2 mL sterilized plastic vials at -80°C . DNA was extracted from frozen sediment samples (200–400 mg) using a previously published protocol based on bead-beating and phenol–chloroform extraction (Griffiths et al. 2000). DNA yields of extractions were determined with Qubit 3.0 Fluorometer and QubitTM dsDNA HS Assay Kit for DNA (Thermo Fisher Scientific). DNA extractions were stored at -20°C before sequencing analyses.

PCR of 16S rRNA genes and amplicon sequencing took place commercially at FISABIO (Valencia, Spain; <https://fisabio.san.gva.es/en/inicio>). For each sample, V3–V4 region of the bacterial and archaeal 16S rRNA genes were simultaneously targeted using primer pair Pro341F (5'-CCTACGGGNGBCAS-CAG-3')/Pro805R (5'-GACTACNVGGGTATC-TAATCC-3') (Takahashi et al. 2014). Archaea were also specifically studied from each sample by targeting the V3–V4 region of the archaeal 16S rRNA genes using primer pair 340F (5'-CCCTAYGGGGYG-CASCAG-3')/806R (5'-GGACTACVSGGGTATC-TAAT-3') (Takai and Horikoshi 2000; Gantner et al. 2011). PCR mixtures (total volume of 25 μL) included approximately 25 ng of template DNA, 12 μL of $2 \times \text{KAPA HiFi HotStart ReadyMix}$ (KK2602,

Roche) and 5 μL of each primer (1 $\mu\text{mol L}^{-1}$). DNA amplicon libraries were generated using a limited cycle PCR: initial denaturation at 95°C for 3 min, followed by 25 cycles of annealing (95°C for 30 s, 55°C for 30 s, 72°C for 30 s) extension at 72°C for 5 min. Thereafter, Illumina sequencing adaptors and dual-index barcodes (Nextera XT index kit v2, FC-131-2001) were added to the amplicons. Libraries were purified with AMPure XP after each amplification. Their DNA content was measured using a Qubit 2.0 Fluorometer and a dsDNA HS Assay Kit (Thermo Fisher). Libraries were normalized and pooled in equal amounts prior to sequencing. The pool containing the indexed amplicons was then loaded onto the MiSeq reagent cartridge v3 (MS-102–3003) and 4 pM of this pool was spiked with 25% PhiX control to improve base calling during sequencing, as recommended by Illumina for amplicon sequencing. Sequencing was conducted using a paired-end 2×300 bp cycle run on an Illumina MiSeq sequencing system.

Bioinformatic analyses

Trimming of the raw data (parameters, min_length: 50; trim_qual_right: 30; trim_qual_type: mean; trim_qual_window: 20) was done using prinseq-lite (Schmieder and Edwards 2011). The paired end reads, R1 and R2, from Illumina sequencing were joined using FLASH program applying default parameters (Magoč and Salzberg 2011).

Mothur (Schloss et al. 2009) was used in subsequent sequence analyses. The sequences were aligned using Silva reference alignment (Release 123). Chimeric sequences, identified using UCHIME (Edgar et al. 2011), were removed from each library and a preclustering algorithm (Huse et al. 2010) was used to reduce the effect of sequencing errors. Sequences were assigned taxonomies with a naïve Bayesian classifier (bootstrap cut-off value 75%) (Wang et al. 2007), using the Silva database (Release 128). Thereafter, sequences classified as chloroplast, mitochondria and eukaryota were removed from each library, while in addition, bacterial sequences were removed from the archaeal V3–V4 library (340F/806R).

Sequences were divided into operational taxonomic units (OTUs) at a 97% similarity level. Singleton OTUs (OTUs with only one sequence) were removed, and the data were then normalized by subsampling to the same size, which was 20,755 for prokaryotic (i.e.

bacterial and archaeal) and 63,673 for archaeal libraries. Good's coverage was over 0.88 and 0.99 in each prokaryotic and archaeal library, respectively, confirming that sequence variation was well covered (data not shown). This study focused specifically on OTUs assigned to known aerobic and anaerobic methanotrophic taxa and on known methanogenic taxa (Nazaries et al. 2013). In addition, as sulfate reducing bacteria are involved in S-AOM and as we also generally wanted to reveal genetic potential for anaerobic sulfate and Fe^{3+} respiration, we also focused on known sulfate and Fe^{3+} reducing taxa (Kuever et al. 2005; Youssef et al. 2009; Kuever 2013; Lovley 2013; Rabus et al. 2013).

Sequence accession numbers

Raw sequencing data has been submitted to National Center for Biotechnology Information's (NCBI) Short Read Archive (SRA) under accession number PRJNA574569.

Results

Porewater biogeochemistry

Profiles of SO_4^{2-} were broadly similar between the three sites (Figs. 2 and 3), though there was a seawards increase in maximum SO_4^{2-} values in the top sediment from 2.9 to 5.0 mmol L^{-1} , from site A to J. Similar to SO_4^{2-} , CH_4 profiles displayed similarity between sites, with key differences (Fig. 3). Lowest concentrations of 0.01 mmol L^{-1} were found at site A surface sediments and highest of 5 mmol L^{-1} at site J below 30 cm depth. At site A, CH_4 was largely absent in the upper sediments, whereas in contrast C was the only site studied with clearly discernible amounts of CH_4 near the sediment–water interface. Overall, CH_4 concentrations displayed a seaward increase, with both absolute concentrations and inventories increasing markedly from site A to J. Site A was the only station that showed a steep decrease in CH_4 concentration below 33 cm depth. All sites display a clear SMTZ, defined as the depth of equal porewater CH_4 and SO_4^{2-} concentrations. The depth of the SMTZ varied between the sites, being located at 13.0, 7.6 and 10.5 cm depth at sites A, C and J, respectively (Fig. 2).

Other biogeochemical parameters were similarly variable between the sites. Porewater Fe concentrations exhibited large variation between the study sites (Fig. 2). Highest concentrations were found at site A, nearest to the river mouth. Surface sediments at site A contained 898 $\mu\text{mol L}^{-1}$ of Fe in porewaters. Concentrations declined rapidly towards the SMTZ, down to 253 $\mu\text{mol L}^{-1}$, after which they increased again reaching 655 $\mu\text{mol L}^{-1}$ at 24 cm depth. Site C showed a slight elevation of Fe in the surface sediment (169 $\mu\text{mol L}^{-1}$), but there, too, concentrations decreased rapidly towards the SMTZ and were largely below the detection limit below it. Below 20 cm depth, the iron concentrations started increasing again. Site J Fe concentrations were low throughout, with only a small elevation of 22 and 23 $\mu\text{mol L}^{-1}$ found at 2 cm depth and below 26 cm depth, respectively.

Porewater profiles of Mn were similarly highly variable between sites (Fig. 2). Site A displayed a mostly flat profile, with slightly lower concentrations near the sediment surface, but ca. 50 $\mu\text{mol L}^{-1}$ concentrations throughout the rest of the sediment column. Site C contained a small peak near the sediment surface, followed by a rapid decrease towards the SMTZ, below which Mn increased again, reaching 196 $\mu\text{mol L}^{-1}$ at 30 cm depth. Site J displayed a similar pattern, except that overall the concentrations were lower, reaching only 69 $\mu\text{mol L}^{-1}$ at 30 cm depth, and the increase did not begin until clearly below the SMTZ.

Calcium profiles were included as a proxy for porewater salinity. Accordingly, the average porewater calcium concentrations increased with distance offshore (Fig. 2). At site A, porewater calcium concentration increased slightly with sediment depth from 1593 to 1715 $\mu\text{mol L}^{-1}$. At site D concentrations varied between 1775 and 2011 $\mu\text{mol L}^{-1}$, decreasing slightly with depth. At site J, concentrations increased slightly with depth and varied between 2392 to 2656 $\mu\text{mol L}^{-1}$.

Methane oxidation rates

CH_4 oxidation activity showed clear and distinct vertical zonation at all three sites (Fig. 3). The activity zones could be divided into three layers: 1. surface-sediment (above the SMTZ), 2. close to the SMTZ, and 3. below the SMTZ. CH_4 oxidation in the surface sediment layer was most evident at site C with a rate of

Fig. 2 Porewater chemistry at the different sampling sites. Note the different scales on the x-axes and the inset of the iron concentration at site J. The grey line represents the depth of the SMTZ at each site, defined as the depth of equal CH₄ and SO₄²⁻ concentrations

$29.52 \pm 7.21 \text{ nmol cm}^{-3} \text{ day}^{-1}$. Site A featured a distinct peak in oxidation activity between the SMTZ and the sediment surface, while at site J oxidation above the SMTZ was low. Oxidation activity near and around the SMTZ was most evident at site J, with a peak of $32.09 \pm 1.69 \text{ nmol cm}^{-3} \text{ day}^{-1}$ found at 11 cm depth, which was also the highest recorded rate throughout the study transect. Site C also featured a very apparent, albeit smaller, increase in oxidation activity near the SMTZ. Clearly elevated oxidation rates were found below the SMTZ at site J, with the highest rate of $13.89 \pm 7.34 \text{ nmol cm}^{-3} \text{ day}^{-1}$ found from 31 cm depth. Site A also showed oxidation activity below the SMTZ, though in lesser degree, whereas at site C oxidation rates drop to near zero in the deepest parts of the core. Standard deviation between replicate oxidation rates was 4.1–93.9%, with an average of 33.97%.

To quantify the lateral (between stations) and vertical differences in CH₄ oxidation rates systematically, we divided each profile into three sections of equal thickness, and calculated depth-integrated rates for each. Full-core integrated rates displayed an overall seaward increase from 2.32 to 3.51 $\text{mmol m}^{-2} \text{ day}^{-1}$ from site A to J (Table 1). At site A, the highest integrated oxidation rate of 0.94 $\text{mmol m}^{-2} \text{ day}^{-1}$ was found at the 1–10 cm depth interval, with a decrease in the 11–20 cm depth interval and a slight increase in the deepest section. In contrast, site C displayed a downwards trend in the integrated rates, decreasing from 0.87 $\text{mmol m}^{-2} \text{ day}^{-1}$ in the topmost section, down to 0.46 $\text{mmol m}^{-2} \text{ day}^{-1}$ in the bottom section. While site J featured the lowest integrated rates of the whole study in the surface section (0.38 $\text{mmol m}^{-2} \text{ day}^{-1}$), in contrast the highest (2.00 $\text{mmol m}^{-2} \text{ day}^{-1}$) and second highest

Fig. 3 Vertical sediment profiles of methane oxidation rates from all sites (three replicates per site shown with data points, mean rate at each depth interval shown with the red line), coupled with dissolved porewater CH_4 and SO_4^{2-} concentrations from the same sites. The grey line denotes the depth of the SMTZ, as defined by the depth of equivalent CH_4 and SO_4^{2-} concentration. Note the different x axes

Table 1 Depth integrated oxidation rates for all sites and depth zones in $\text{mmol m}^{-2} \text{ day}^{-1}$

Depth	A	C	J
1–10 cm	0.94	0.87	0.38
11–20 cm	0.61	0.82	2.00
21–31 cm	0.77	0.46	1.13
Total	2.32	2.16	3.51

Highest values of each depth zone across all sites in bold typeface

($1.13 \text{ mmol m}^{-2} \text{ day}^{-1}$) integrated rates of the whole study were found from the 11–20 cm and 21–31 cm depth zones respectively.

Microbial community structure

A clear vertical structure was observed in the microbial community composition at sites A and D as determined by relative abundances of 16S ribosomal RNA genes (Figs. 4 and 5, Supplementary Figs. 1 and 2). The dominant archaeal taxa in both sites were *Methanomicrobia* and *Thermoplasmata* (within *Euryarchaeota*) as well as *Bathyarchaeota*, *Thaumarchaeota* and *Woeisearchaeota* (Supplementary Fig. 1). The bacterial community was more diverse than the archaeal community. The most dominant bacterial taxa in both sites included e.g. *Deltaproteobacteria*, *Gammaproteobacteria* and *Betaproteobacteria* (within *Proteobacteria*) as well as *Bacteroidetes*, *Chloroflexi* and *Verrucomicrobia* (Supplementary Fig. 2). In the following, we report the relative abundance of microbial groups associated with specific CH_4 -related processes and with sulfate and Fe^{3+} reduction within this vertical structure, as well as differences between sites A and D. Since the microbial data is derived from cores sampled in June 2015, we report the depth of the SMTZ as determined from these cores by Jilbert et al. (2018), rather than from the 2017 profiles shown in Figs. 2 and 3.

Vertical structure of methane-related as well as sulfate and Fe^{3+} reducing microbial communities

The relative abundances of taxa associated with AOM, MOX and MOG showed a clear vertical zonation at both sites (Fig. 4k–n). MOX-related taxa dominate in

the surface sediments, while AOM and MOG taxa became more dominant with increasing depth in the sediment column. The contrasting depth of the SMTZ at these two sites is reflected in the depth of the switch from MOX-to AOM-dominance of the CH_4 oxidizer community, which occurs deeper at site D (Fig. 4m–n). Below the SMTZ, AOM-related groups show a similar abundance to methanogens at site D, while at site A, methanogens dominate over AOM-related groups throughout the deeper sediments (Fig. 4k–l). Putative sulfate and Fe^{3+} reducing bacteria were present all through the sediment columns at both sites (Fig. 5). The vertical variations in the total relative abundance of Fe^{3+} reducers were quite minor at both sites, yet at site A they were highest in the surface above SMTZ and in the bottom layers, while multiple minor peaks in their relative abundance were observed along the sediment column in site D (Fig. 5). In contrast, sulfate reducers had lower relative abundance at the surface layers and at SMTZ than deeper in the sediment column at site A, while at site D they had highest relative abundance at the surface layers above the SMTZ and decreased drastically in deeper layers especially from 30 to 40 cm depth (Fig. 5).

Groups associated with AOM

From the microbial groups commonly associated with AOM, ANME-2a/b was the most abundant at both sites. The relative abundance of ANME-2a/b generally increased with depth in the sediment column (Fig. 4a–d). When reported as a fraction of total prokaryotes, relative abundances of this group were thus highest below the SMTZ at both sites (Fig. 4c–d). This effect was most pronounced at site D, where ANME-2a/b also dominated AOM-related groups (Fig. 4d). At site A, high relative abundances of ANME-2a/b were also observed in the uppermost 10 cm, broadly within the SMTZ at this site (Fig. 4a). ANME-2d contributed an additional important group at site A, with a clear increase below the SMTZ (Fig. 4a, c). At site D, ANME-2d was present in small numbers (0.01–0.32%) in the archaeal community throughout the sediment column (Fig. 4b). ANME-1 was present in even smaller numbers at throughout the sediment column at site A (0.002–0.009% of archaeal 16S rRNA genes) but entirely absent at site D (Fig. 4a–b). *Ca. Methyloirabilis* (*NC10* phylum) was also present in very small numbers (0.002–0.005% of 16S

Fig. 4 Relative abundances of different microbial groups (AOM = clades associated with AOM, MOG = clades of methanogens, MOX = clades of bacteria capable of MOX, TOTAL = groups summed up) at sites A and D in June 2015. Note the different scales on the x-axes for archaea (columns I–

II) and prokaryotes (III–IV). Abundances of archaea are reported as a fraction of total archaea and abundances of prokaryotes reported as a fraction of total prokaryotes (archaea + bacteria). The grey line represents the depth of the SMTZ in June 2015 (Jilbert et al. 2018)

prokaryotic rRNA genes at 4–12 cm depth) and only at site A, with the highest abundance of 0.010% found from 20 cm depth (Fig. 4c).

Groups associated with MOX

Methylococcales (Type I) were the most abundant group of MOB in surface sediment at both sites, remaining more abundant deeper in the sediment at site A (Fig. 4i). However, their abundance decreased rapidly below the SMTZ. *Methylocystaceae* (Type II) MOB showed two peaks in abundance below the SMTZ at site A, but were largely absent from site D (Fig. 4i–j). MOB belonging to the phylum *Verrucomicrobia*, i.e. *Ca. Methylacidiphilum* sp., were present in small numbers in the surface sediment of both sites.

Groups associated with methanogenesis (MOG)

Methanomicrobiales, *Methanosaetaceae* and *Methanosarcinaceae* were the most abundant methanogen taxa at both sites, while other methanogens, i.e. *Methermicrococcaceae*, *Methanobacteria*, *Methanocellales* and *Methanomassiliicoccus* sp. were scarce (Fig. 4). *Methanomicrobiales*, which consisted dominantly of *Methanoregulaceae*, were the most abundant at site A and made up to 20% of all archaea at 35 cm depth (Fig. 4e), while the MOG community at site D was less abundant but more varied in distribution between *Methanomicrobiales*, *Methanosaetaceae* and *Methanosarcinaceae* (Fig. 4f). At both sites, the relative abundance of all the dominant taxa of methanogens generally increased below the SMTZ. However, at site D the largest increase was observed deeper in the sediment column (Fig. 4h).

Fig. 5 Relative abundances of dominant microbial taxa and sum of relative abundances of all microbial taxa putatively involved in sulfate and Fe^{3+} reduction. Relative abundances are presented as a fraction of total prokaryotes (archaea + bacteria)

Groups associated with sulfate and Fe^{3+} reduction

The relative abundance of sulfate reducers was higher at site D than A. Sulfate reducers were also more abundant than Fe^{3+} reducers at both sites (Fig. 5). The putative sulfate reducing community in both sites consisted mostly of *Desulfobacterales* and *Desulfarculaceae* with minor contribution also from *Desulfobacca* sp., *Desulfomonile* sp., *Desulfosporosinus* sp. and *Desulfovibrionales* (Fig. 5). *Desulfobacterales* and *Desulfarculaceae* were generally of similar relative abundance at site A, except at surface layers and at SMTZ, where *Desulfobacterales* were at their highest and *Desulfarculaceae* at their lowest levels (Fig. 5). In contrast, in site D, *Desulfobacterales* significantly outnumbered *Desulfarculaceae* at all layers and generally followed the same depth pattern as total sulfate reducing community, while the relative abundance of *Desulfarculaceae* was quite stable through the sediment column (Fig. 5).

The putative Fe^{3+} reducing bacterial community in both sites consisted mostly of *Deferribacteres*, *Geobacteraceae* and *Thiobacillus* with minor contribution also from *Geothrix* sp., *Pseudomonas* sp., *Thermotogae*, *Bacillus* sp., *Desulfuromonas* sp., *Shewanella* sp., *Ferribacterium* sp. and *Aeromonas* sp. (Fig. 5). The dominant taxa differed in their vertical distribution patterns. *Geobacteraceae* generally had

highest relative abundance at surface layers above SMTZ, whereas *Deferribacteres* were more abundant at deeper layers (Fig. 5).

Discussion

Vertical structure of methane oxidation rates in the context of porewater data

The oxidation rates measured in this study (Fig. 3) are broadly comparable to those of previous studies in the Baltic Sea, e.g. Thang et al. (2013) reported maximum rates of $16 \text{ nmol cm}^{-3} \text{ day}^{-1}$ from Sweden (Himmerfjärden bay) and Treude et al. (2005) reported rates of $1\text{--}14 \text{ nmol cm}^{-3} \text{ day}^{-1}$ in the upper 20 cm of the sediment in Germany (Eckernförde bay). However, while the rate profiles of Thang et al. and Treude et al. typically contained only a single clear peak of oxidation near the SMTZ, the oxidation rate profiles in this study can broadly be divided into three distinct layers: 1. surface-sediment (above the SMTZ), 2. close to the SMTZ, and 3. below the SMTZ. Oxidation in each layer was most likely mediated by different EAs. In layer 1, above the SMTZ, and especially close to the sediment surface, the observed oxidation was most likely related to MOX (Osudar et al. 2015; Steinle et al. 2017). In layer 2, the steep converging CH_4 and

SO_4^{2-} gradients, forming a well-defined SMTZ at all three sites, strongly suggest S-AOM at this depth (Iversen and Jørgensen 1985). Indeed, the most conspicuous peaks in oxidation at sites C and J are observed at the SMTZ (Fig. 3). However, we also measured oxidation activity in layer 3 at all sites, well below the SMTZ, at depths where porewater SO_4^{2-} was completely depleted. Therefore, we must attribute this activity to AOM mediated by other EAs than SO_4^{2-} , most likely by oxides of either Fe (Egger et al. 2015; Rooze et al. 2016) or Mn (Beal et al. 2009). The porewater profiles show evidence for reduction of Fe and Mn oxides below the SMTZ at all sites (Fig. 2). Accumulation of porewater Fe is most apparent at site A, while accumulation of Mn is most apparent at site C, likely reflecting the contrasting availability of Fe and Mn oxides at these two sites.

Lateral variability in methane oxidation along transect

One key finding of the study is that the maximum depth-integrated rates of CH_4 oxidation at any site were observed at site J. Moreover, this site also showed higher rates of oxidation in layer 3 (below the SMTZ), with respect to the other sites (Table 1). This observation suggests that non- SO_4^{2-} AOM may be an important biogeochemical process in the archipelago regions of the northern Baltic Sea, and apparently more prevalent in the archipelago sediments than in the low-salinity estuaries that drain into these areas. This conclusion appears surprising, since the availability of Fe and Mn oxides is greater in the inner estuarine areas (sites A and C in this study), and the porewater data from these sites suggest more active reduction of oxides. However, we note that there are several reasons why the accumulation of porewater Fe^{2+} and Mn^{2+} may not directly reflect rates of Fe- and Mn-AOM. Principally, dissimilatory reduction of oxides coupled to organic matter respiration may also generate these dissolved species (e.g. Egger et al. 2015). Also, the presence of excess porewater H_2S at the SMTZ will draw down their concentrations through precipitation of sulphide minerals (e.g. Reed et al. 2011). Jilbert et al. (2018) showed that site J is characterized by a strongly developed H_2S maximum at the SMTZ, which could explain the relatively low porewater Fe and Mn accumulation at this site despite high rates of AOM.

We therefore suggest that rates of non- SO_4^{2-} AOM are not controlled by EA availability in our study transect. Apparently, all the studied sites have sufficient Fe and/or Mn oxides in the sediments to facilitate AOM below the depth of SO_4^{2-} penetration. Rather, the availability of CH_4 (i.e. the rate of methanogenesis) controls the observed rate of non- SO_4^{2-} AOM at any given site. Depth-integrated oxidation rates below the SMTZ were highest at site J (Fig. 3), which is also characterized by the highest porewater CH_4 concentrations (Fig. 2). The reason for the high porewater CH_4 at site J is likely related to the high accumulation rate of degradable organic material at this location. Rates of CH_4 production in the 12–20 cm interval of the sediment column at site J have been estimated from porewater profiles as $8\text{--}10 \text{ nmol cm}^{-3} \text{ day}^{-1}$ (Jilbert et al. 2018). That study showed that the sediments of the archipelago are dominated by phytoplankton-derived organic carbon, while the inner estuarine sites have a mixed phytoplankton-terrestrial organic matter composition. Hence, for an equivalent total accumulation rate of carbon, archipelago areas are likely to have higher overall rates of remineralization reactions—including methanogenesis—due to the relative lability of the carbon source (Arndt et al. 2013).

Site-specific characteristics in the oxidation rate profiles

Due to the presence of oxygen in bottom waters, we expected to find evidence of MOX in the surface sediments at all sites (Fenchel et al. 1995). In addition, we expected to find the surface sediment at the nearly freshwater site A to contain the highest oxidation rates of the three sites. This is motivated by previous observations that MOX has a strong inverse relationship with salinity (de Angelis and Scranton 1993), likely caused by changes in the relative availability of oxygen, sulfate and methane along salinity gradients. However, while the integrated rates in the surface sediment layer were indeed highest at site A (Table 1), it lacked the distinct oxidation peak seen especially at site C and to a lesser degree at site J (Fig. 3).

We suggest that the differences in oxidation activity in the surface sediments can again be explained partly by CH_4 availability: site C was the only site to contain appreciable amounts of porewater CH_4 close to the sediment–water interface, and accordingly displayed

the shallowest SMTZ of the three sites (Fig. 2). Also, given that sites A and J are consistently oxic, strong bioturbation is expected (Vaquer-Sunyer and Duarte 2008). This may blur the diagenetic zones, allowing MOX to co-occur with S-AOM in the vicinity of burrows and hence to obscure the development of a distinct double-peak structure in the oxidation rate profile.

All three sites featured distinct oxidation maxima near the depth of SMTZ, interpreted as S-AOM, though the peak became more pronounced offshore (Fig. 2). Integrated oxidation rates in layer 2 (close to the SMTZ) increased considerably offshore and highest integrated rates of the whole study were found at site J in this layer (Table 1). The increased rates of S-AOM offshore are likely a reflection of dual substrate availability. S-AOM is dependent on both the availability of CH_4 and SO_4^{2-} (Valentine 2002). As outlined above, CH_4 concentrations are highest at site J due to organic matter degradability. Moreover, due to higher bottom water salinity, SO_4^{2-} availability is also greater at this site.

Microbial communities at the study sites

The sediment archaeal and bacterial communities at sites A and D generally consisted of the same taxa as in coastal and offshore sites of another northern Baltic Sea area, the Bothnian Sea (Rasigraf et al. 2019) (Supplementary Figs. 1 and 2). Furthermore, the same dominant methanogen taxa, *Methanomicrobiales* (*Methanoregulaceae*), *Methanosaetaceae* and *Methanosarcinaceae*, were present both at our study sites and in Bothnian Sea sediments (Rasigraf et al. 2019) (Fig. 4). Although Rasigraf et al. (2019) were able to construct a metagenome-assembled-genome of *Methanomassiliicoccaceae* thriving in Bothnian Sea sediments, this family, alongside other methanogenic taxa, were at very low levels both at ours and their study sites. *Methanomicrobiales* and *Methanosaetaceae* are considered to drive hydrogenotrophic and acetoclastic methanogenic pathways, respectively, while both acetoclastic and methylotrophic pathways have been suggested for *Methanosarcinaceae* (Nazaries et al. 2013). However, in environments with Fe^{3+} reducing bacteria and conductive minerals such as in iron-rich sediments of our study sites, *Methanosaetaceae* and *Methanosarcinaceae* could also drive CO_2 – reduction (to CH_4) coupled with either direct or

indirect (via conductive materials) electron transfer from iron-reducing bacteria, such as *Geobacter* (Rotaru et al. 2014, 2018). Some metagenomic datasets also indicate *Bathyarchaeota*, which were dominant archaea both at our study sites and Bothnian Sea sediments (Rasigraf et al. 2019) (Supplementary Fig. 1), can be both methanogenic and methanotrophic (Evans et al. 2015). Shotgun metagenomic data would be needed to assess genetic potential of *Bathyarchaeota* at our study sites. However, the metagenomes of Rasigraf et al. (2019) suggest a non-methanogenic/non-methanotrophic fermentative lifestyle for *Bathyarchaeota* in Bothnian Sea sediments.

Genetic potential for sulfate and Fe^{3+} reduction was present throughout the sediment columns based on distribution of sulfate and Fe^{3+} reducing bacteria (Fig. 5). Because many of the Fe^{3+} reducing bacteria can also reduce Mn^{4+} , genetic potential for Mn^{4+} reduction was also present (Lovley 2013). The higher relative abundance of sulfate reducers at site D very likely represented higher marine influence, thus higher availability of sulfate (Fig. 5). Quite surprisingly, except for *Desulfobacterales* at site A, the relative abundance of sulfate reducers was not highest at the SMTZ. In addition, sulfate reducers were present in significant relative abundance also in deeper sulfate-depleted layers (Fig. 5). These deviations can be explained by sulfate reducing bacteria having also alternative metabolism strategies. For example, many sulfate reducers can switch to fermentation if sulfate is depleted (Rabus et al. 2013). We acknowledge that the deviations can also represent a bias in functional grouping, since this was based mostly on data from isolated bacteria. Modern metagenomic studies have shown that potential for both sulfate and Fe^{3+} reduction is spread also beyond the taxa from which isolates exist (Rasigraf et al. 2019; Garber et al. 2020).

Microbial community data in the context of oxidation rate data

Our theory that availability of CH_4 was the key factor controlling the observed oxidation rates is supported by the microbial data. At both stations sampled for microbial communities, MOG generally occur concurrently with ANME (Fig. 4k–n), implying that AOM co-occurs with methanogenesis regardless of depth in the sediment column. Importantly, both MOG

and ANME taxa increase in abundance below the SMTZ at both sites (Fig. 4m–n). There is also significant overlap in the presence of MOB and ANME, especially at site A, and MOB are found in much greater abundances deeper in the sediment than at site D (Fig. 4m–n). This also supports our previous conclusions regarding the potential effects of bioturbation and the blurring of diagenetic zones above the SMTZ.

Of the AOM related clades included in this study, ANME-1 is generally considered the least flexible in terms of EAs and is mainly associated with S-AOM (Timmers et al. 2017). By contrast, ANME-2a/b have been shown to be capable of both S-AOM and Me-AOM (Timmers et al. 2017), and ANME-2d is suggested to be capable of at least S-AOM, N-AOM, Fe-AOM and Mn-AOM (Haroon et al. 2013; Ettwig et al. 2016; Timmers et al. 2017). The presence of sulfate and Fe^{3+} reducing (and Mn^{4+} reducing) bacteria throughout the sediment columns further support the role of sulfate reducers as bacterial partners in S-AOM and also more generally the role of SO_4^{2-} and Fe^{3+} (and Mn^{4+}) as electron acceptors in anaerobic processes. The dominance of ANME 2a/b in our data likely reflects the mixed-EA environment of the estuarine system in general, with relatively low SO_4^{2-} and high oxide availability when compared to fully marine settings. Treude et al. (2005) and Rasigraf et al. (2019) also found that in Eckernförde bay (southern Baltic) and Bothnian Sea (northern Baltic), respectively, which also feature relatively shallow SMTZs, ANME-2 were the dominant clade. ANME-2 have also been shown to dominate other shallow SMTZ sediments in the ocean (Losekann et al. 2007).

ANME-2d has previously been found the most abundant AOM clade in some lakes (Weber et al. 2017; Rissanen et al. 2017). We also observed the highest abundances of ANME-2d at the river mouth-site, while at the slightly more saline site D, ANME-2d was still present, but in much lower abundances (Fig. 4a–d). Thus, despite multiple EAs that ANME-2d could potentially utilize (Timmers et al. 2017), it seems that salinity strongly inhibits the presence of ANME-2d, reinforcing previous interpretations of this as a primarily freshwater clade (Timmers et al. 2017).

The anaerobic taxa *Ca. Methyloirablis* (NC10 phyla), also capable of N-AOM (Welte et al. 2016), was also present in our study, but only at site A (Fig. 4c, d). These taxa has previously been found

from both freshwater and marine environments (Welte et al. 2016). Its environmental significance remains unknown, as some studies have found no significant contributions from bacteria of the NC10 phyla to CH_4 oxidation (Beck et al. 2013; Martinez-Cruz et al. 2018), while others have shown the clade capable of very active nitrite N-AOM in lakes, though only in the first few millimeters of the sediment surface (Deutzmann et al. 2014). In our study it was most abundant relatively deep (20 cm) in the sediment, though we have no way of showing whether it was active at those depths. Nevertheless, it is possible that bioirrigation may introduce nitrite deeper in the sediment and enable N-AOM to occur there intermittently. This, too, could further contribute to the blurring of the diagenetic zones as discussed previously, as Deutzmann et al. (2014) also note that N-AOM by the NC10 bacteria could potentially be confused with MOX.

The MOB present in the surface sediment of site A were predominantly type I, which was to be expected as they are considered to be most common in estuaries (Dean et al. 2018). However, there was also a clear enrichment of type II MOB below the SMTZ (Fig. 4i). The co-occurrence of aerobic and anaerobic methanotrophs has previously been observed in lakes (Deutzmann et al. 2014; Martinez-Cruz et al. 2018). While it is possible that MOX occurs below the SMTZ at site A due to bioturbation, it is unlikely. More likely, the presence of type II MOB deeper in the sediments is the combined result of burial due to intensive sedimentation and selective survival (Rissanen et al. 2019). Indeed, Type II MOB have been shown to be able to survive for extended periods in a dormant state and recover CH_4 oxidation capability even after decades of anoxic conditions (Roslev and King 1994).

Alternative interpretations of oxidation pathways

Here we present a number of alternative potential biogeochemical pathways that could lead to the observed diagenetic zonation, and assess the likelihood that they may play a role in our study system.

All the sites studied here have been previously shown to accumulate porewater Fe^{2+} below the SMTZ, which we interpret to be linked to Fe-AOM and organoclastic Fe reduction (Jilbert et al. 2018). However, such accumulation has also been postulated to reflect the “cryptic sulphur cycle” (Holmkvist et al. 2011). While it is possible that this process is taking

place in this location, it is most likely confined to the vicinity of the SMTZ or immediately below. H₂S is required to sustain the cryptic sulphur cycle, and this is rapidly depleted below the SMTZ at these sites (Jilbert et al. 2018).

AOM coupled to denitrification (N-AOM) has been shown to be potentially an even more energetically favorable pathway than Me-AOM (Raghoebarsing et al. 2006; Ettwig et al. 2010). This process is expected to be confined to the oxic/anoxic interface in the sediment, which in our study area is so close to the sediment surface that the sampling resolution used is insufficient to capture it. However, as indicated above, given the potential for bioturbation and the presence of *Ca. Methyloirablis* (NC10 phyla) and ANME-2d, it is possible that N-AOM is also taking place at least at site A.

Methanogens are capable of also oxidizing CH₄, while on the other hand methanotrophs are capable of reducing CO₂ to CH₄ (Timmers et al. 2017). This potentially creates a “cryptic CH₄” cycle, in which CH₄ is being oxidized and produced concurrently. These processes may be mediated partly by the same microbes or via syntrophic couplings, both within the SMTZ (Beulig et al. 2019) and shallower in the sediment column (Maltby et al. 2016; Xiao et al. 2017). While we cannot rule out these processes being present from the SMTZ upwards, no evidence yet exists of such processes deeper in the sediments in conjunction with Me-AOM.

Implications and conclusions

Our estimates for CH₄ oxidation rates are broadly similar in magnitude to rates of CH₄ production in sediments from the Pojo Bay estuary (Jilbert et al. 2018; Myllykangas et al. 2020). Thus, our results suggest that sedimentary CH₄ oxidation filters in this estuary and its connected archipelago are functioning efficiently and removing the vast majority of the produced CH₄ in situ, preventing its escape to the water column. This is supported by similarities in the abundances of methanogenic and methanotrophic microbial taxa in sediment profiles, indicating strong co-occurrence of these processes.

We identify three distinct layers of oxidation activity each contributing to the function of the oxidative filter; MOX, S-AOM and Me-AOM. Me-

AOM below the SMTZ is clearly very active throughout the transect, with maximum rates observed at site J. Similarly, S-AOM rates are highest in this site. Overall it is likely the increased availability of CH₄ caused the total integrated rates of both processes to increase offshore. Similarly to the oxidation rates, in the microbial community structure, ANME-2a/b were relatively most abundant below the SMTZ, especially in higher salinity conditions whereas MOB and methanogens were more abundant in the lower salinity.

In many coastal systems, rates of both methanogenesis and methanotrophy have increased as a consequence of anthropogenic eutrophication (Borges et al. 2018). As well as potential impacts on CH₄ fluxes to the atmosphere, the links between CH₄ processes and other elemental cycles may lead to broader impacts on coastal sediment biogeochemistry. Our study shows that non-SO₄²⁻ AOM is important in coastal sediments of the northern Baltic Sea, highlighting in particular the coupling between CH₄ and Fe cycling. The Fe-AOM model of Rooze et al. (2016) found that only 9% of all CH₄ oxidation was performed by Fe-AOM. However, according to their results Fe-AOM had a significant role in Fe cycling, accounting for 46% of all Fe(OH)₃ reduction. Our study suggests that in coastal areas, the role of Fe-AOM may be even greater, leading to even stronger impacts on Fe cycling. This could have further downstream effects on other biogeochemical cycles, such as phosphorus transport to deep basins of the Baltic (Jilbert and Slomp 2013).

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